

**Intermountain Forest Tree  
Nutrition Co-op  
and the  
USDA Forest Service, RMRS  
*Armillaria* root disease study**

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# Review of *Armillaria* species in North America

*Armillaria* can be divided into 3 classifications

"Bad" *Armillaria*

NABS I and NABS VI

primary pathogens

"Good" *Armillaria*

NABS III, V, VII, X, and XI

primary saprophytes

Unknown

NABS II and NABS IX

pathogenicity

(NABS = North American Biological Species)

*Armillaria* species found on Nutrition Co-op plots throughout the Inland Northwestern United States.

"Bad" *Armillaria* - primary pathogen  
NABS I – *Armillaria ostoyae*

- Common
- Known as a highly virulent pathogen of conifers
- Causes tree mortality
- Causes tree growth loss (often with no apparent visible symptoms)



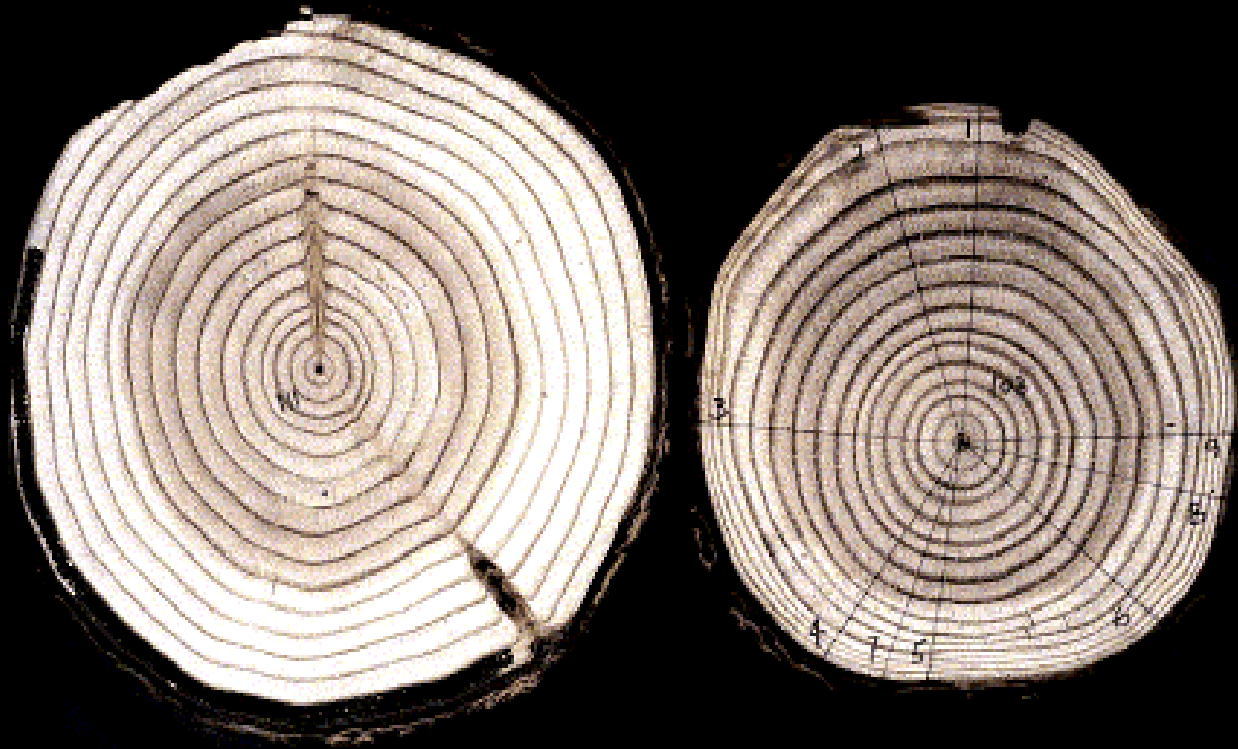
# Example of *Armillaria ostoyae* mortality centers





Average mortality rates for sites covering Northern Idaho, northeastern Oregon, and Eastern Washington vary from 8.8% for Douglas-fir, 4.2% for grand fir, and 1.5% for redcedar habitat types based on 8 sites (Moore et al. 2000).

Growth loss and mortality due to *Armillaria* in British Columbia have been cited as between 1.4 and 3.8 million m<sup>3</sup> annually (White and Morrison, 1999).



The effects of root disease on annual ring width.  
(Cruickshank et al. 2001)

Volume loss has been shown as high as 40% over 4-8 years  
in eighteen-year-old Douglas-fir. (Cruickshank, 2000)



**Resinosus**

**a sign of**

***Armillaria ostoyae***

**infection**

# Unknown pathogenicity

## NABS IX – *Armillaria nabsnona*

- Rare
- Found on wet sites
- Unknown ecological effects



# *Armillaria* species found on nutrition co-op plots throughout the Inland Northwestern United States.

"Good" *Armillaria* – primary saprophytes

NABS III – *Armillaria calvescens*

NABS V – *Armillaria sinapina*

NABS VII – *Armillaria gallica*

- Closely related species
- Common
- Low pathogenicity; 5-10% found as bark fans
- Most individuals show no sign of pathogenicity and may be beneficial for tree growth and or protection.
- Difficult to identify

"Good" *Armillaria* – primary saprophyte  
NABS X – currently unnamed

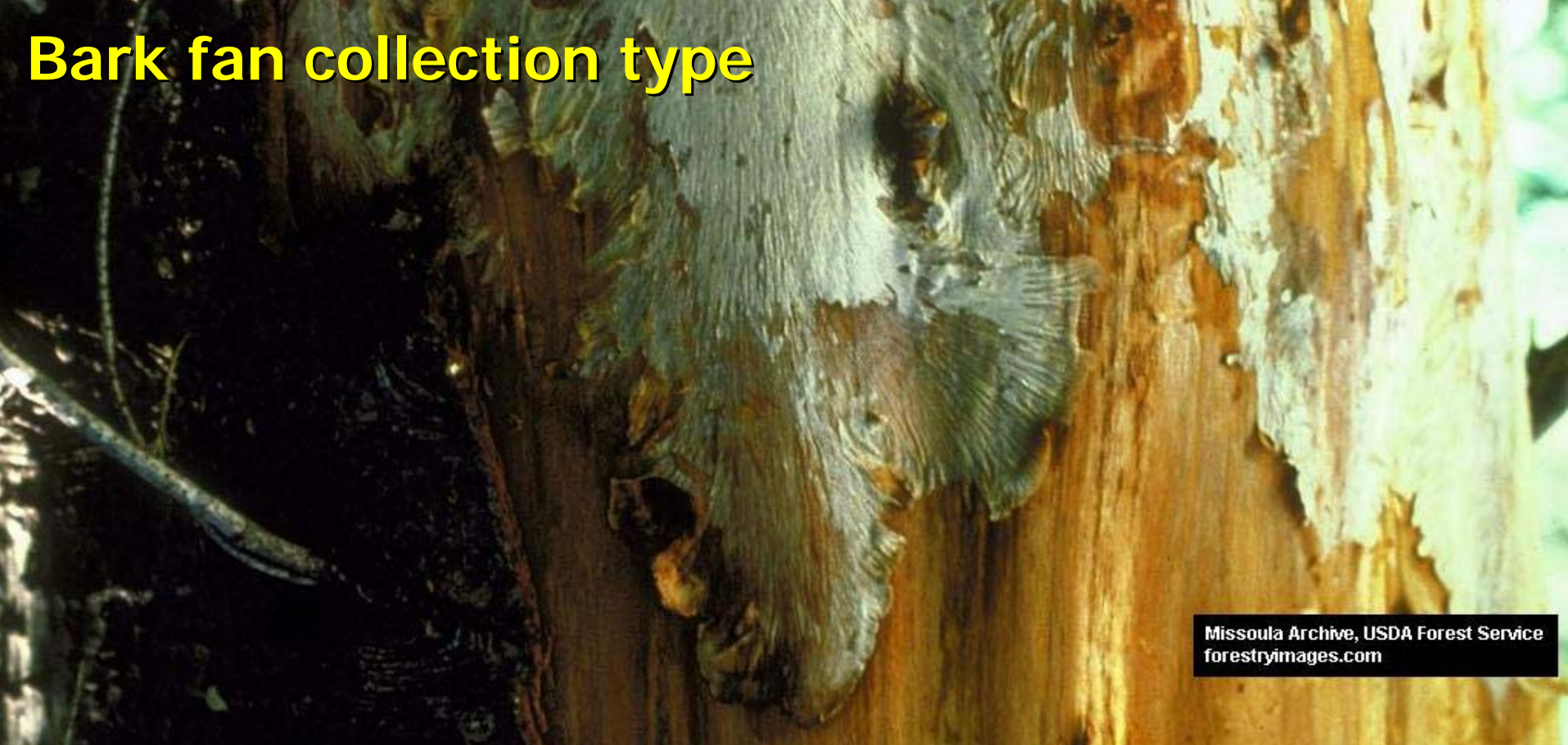
- Common
- Low pathogenicity; 5-10% found as bark fans
- Most individuals show no sign of pathogenicity and may be beneficial for tree growth and or protection.

1,310 *Armillaria* samples were collected from Nutrition Co-op plots during the summers of 1999 and 2000.

Three collection types were taken

- Bark fan
- Rhizomorph
  - Wood

## Bark fan collection type



Missoula Archive, USDA Forest Service  
forestryimages.com

Bark fans from live trees indicate high pathogenicity of an *Armillaria* individual.

Dead trees with bark fans do not necessarily indicate a pathogenic *Armillaria* individual.



# Rhizomorph collection type

Robert L. Anderson, USDA Forest Service  
forestryimages.com



All species of *Armillaria* form rhizomorphs.

Most rhizomorphs are found on the surface of a root, some grow freely through the soil, and others can be found under the bark of a highly infected tree.

Presence of rhizomorphs does not necessarily indicate pathogenicity of an *Armillaria* individual.



# Wood collection type

Robert L. Anderson, USDA Forest Service  
forestryimages.com



Wood samples are usually taken from trees that have recently been killed or are infected with a pathogenic *Armillaria* individual.

Often dark lines known as zone lines can be seen in infected wood.





**Other collection types include caps and spores.**

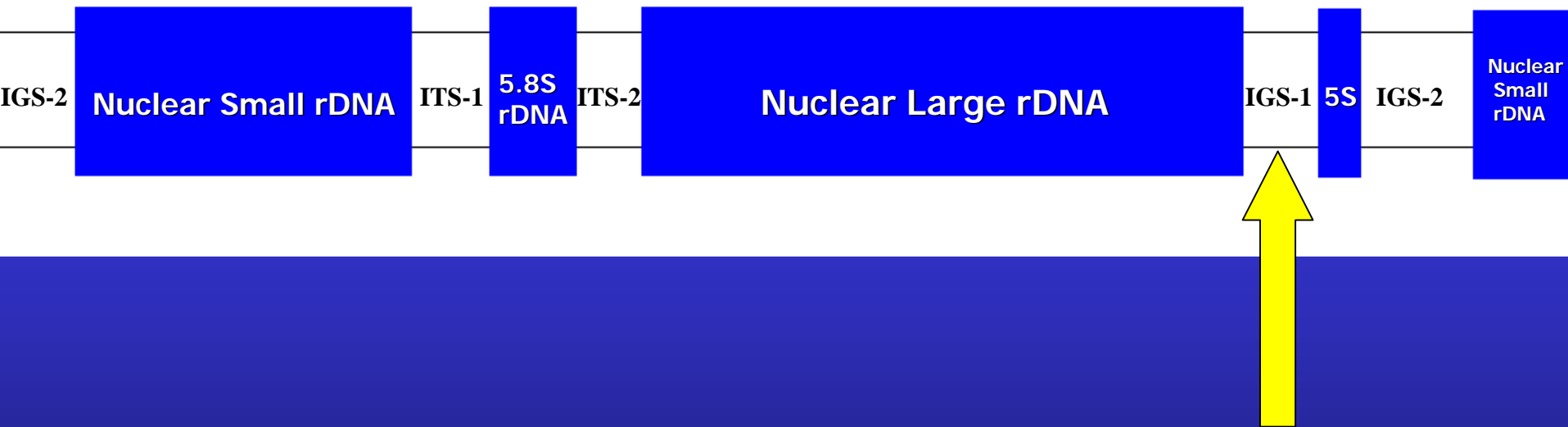
# How do we identify *Armillaria* to species?

- From our collections we obtain pure fungal cultures known as isolates.
- Each isolate is paired against one another in a somatic incompatibility test to determine if it is from the same individual (genet).
- Isolates of the same genet will fuse together, while a distinct zone is formed between isolates of different genets.
- Some genets have been known to cover an area as large as 900 ha, one having an estimated age of 2,400 years old. (Ferguson et al. 2000)



- The fungal isolates grow slowly, and it usually requires several months to run all the somatic incompatibility tests to reduce isolates to individual genets.
- Once the genets are obtained we use Polymerase Chain Reaction (PCR) to obtain multiple copies of a specific region of DNA that we use for genetic identification.
- The region of choice for our studies was the IGS-1 region of rDNA. This region repeats many times throughout an individual's DNA. PCR is used to amplify all the repeats of this region. The IGS-1 has a significant amount of variation between species for identification and it also displays variation within species.

# These regions of rDNA repeat many times

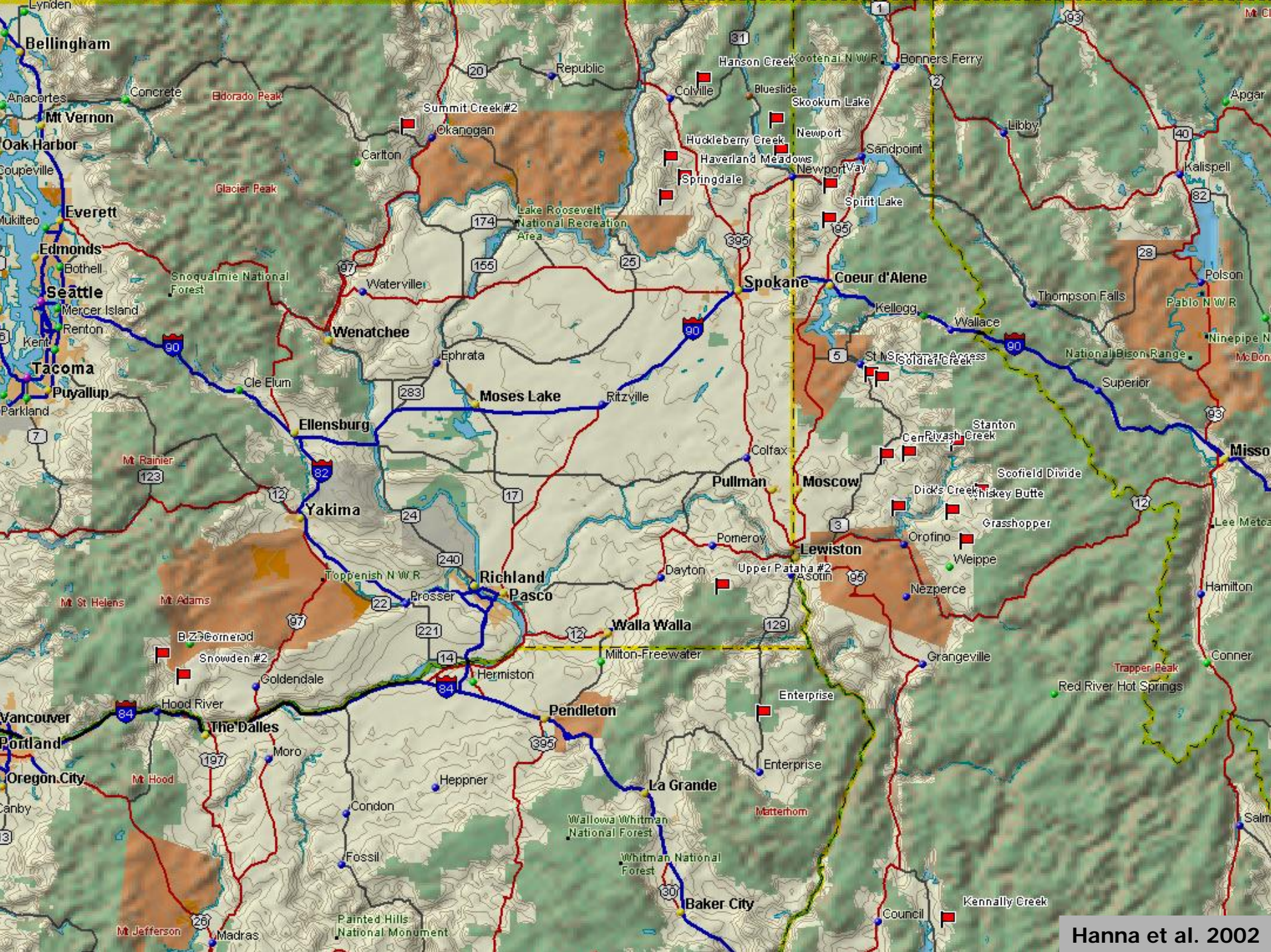


We amplify all the repeats of the IGS-1 region for a given isolate, then we obtain an IGS-1 region sequence











# 1999 and 2000 Nutrition Co-op genets by Species

221 Genets

99 NABS X

62 NABS I

59 NABS III, V, or VII

1 NABS IX

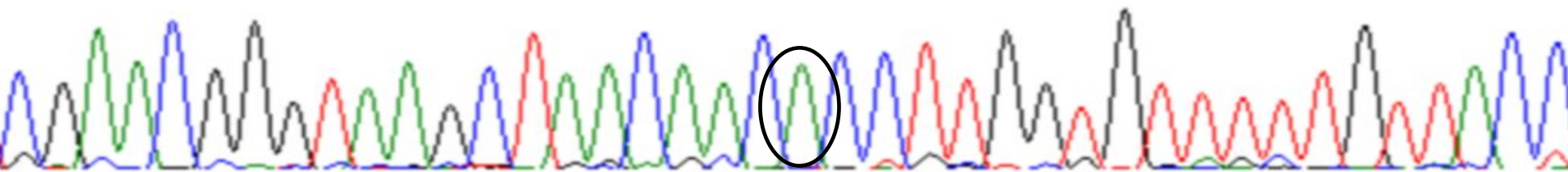
# Phylogenetic tree showing genetic diversity of *Armillaria ostoyae*



# Genetic diversity within *Armillaria ostoyae*

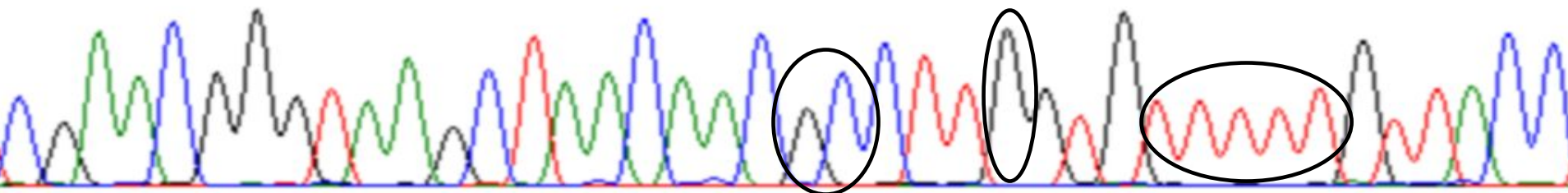
C GAAC GGG TAAGC TAACAACAC CTTGGT GTTTTT GTTACC

**NABS I Group B**



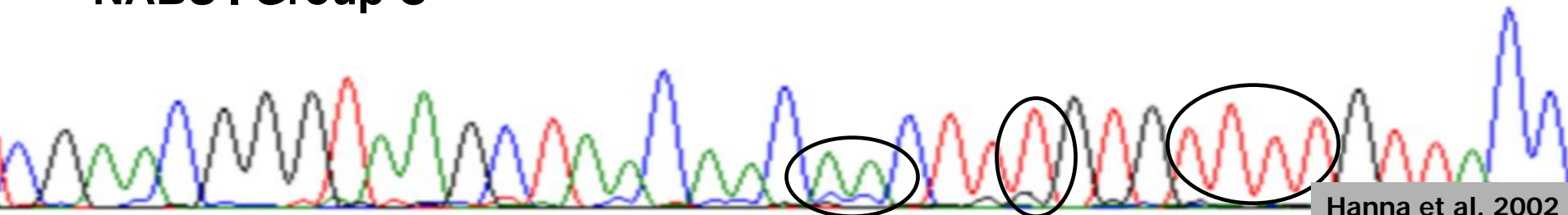
C GAAC GGG TAAGC TAACAAC GC CTTGGT GTTTTT GTTACC

**NABS I Group A**



C GAAC GGG TAAGC TAACAACAAC TTTGT GTTTTT GTTACC

**NABS I Group C**



**Hybridization also found within groups  
of *Armillaria ostoyae*.**

**Hybridization within species is known as  
intraspecific hybridization.**

**NABS I Groups A x B hybrid**

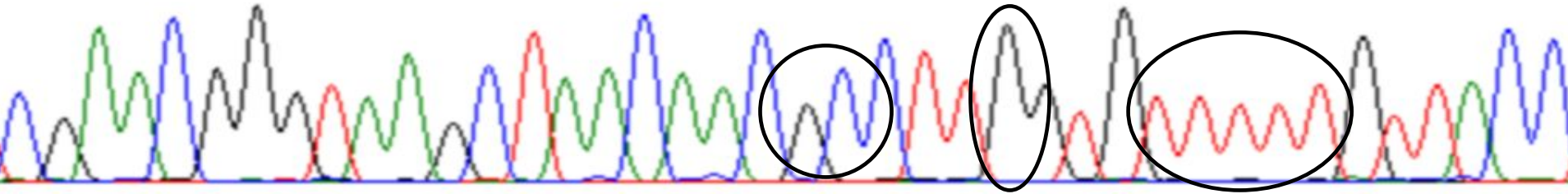
**NABS I Groups A x C hybrid**



# *Armillaria ostoyae* intraspecific hybridization

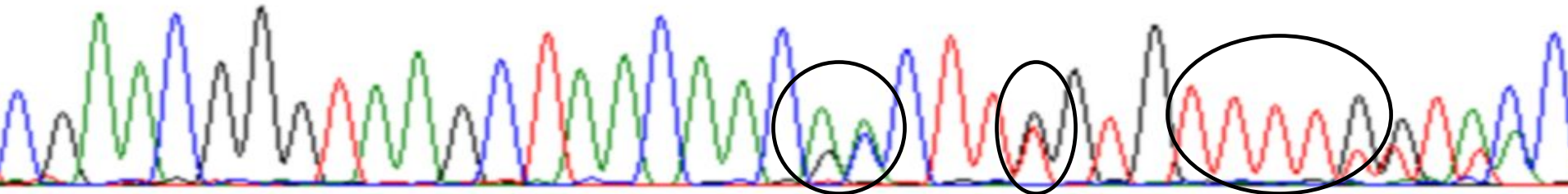
C G A A C G G G T A A G C T A A C A A C G C C T T G G T G T T T T T G T T A C C

**NABS I Group A**



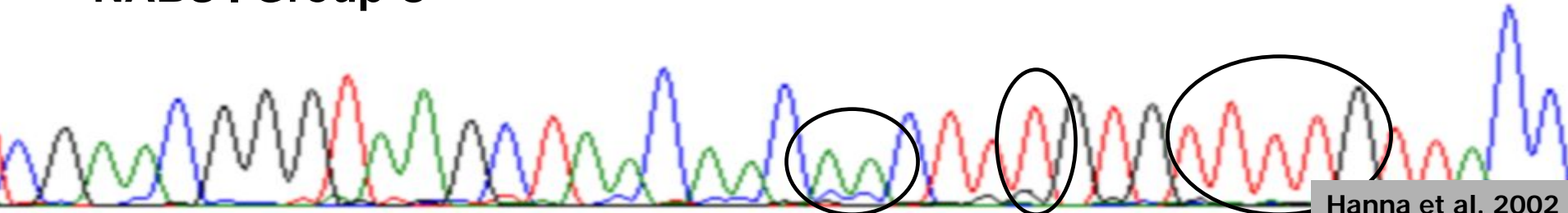
C G A A C G G G T A A G C T A A C A A C A N C T T N G T G T T T T G G T A C C

**NABS I Groups A x C hybrid**



C G A A C G G G T A A G C T A A C A A C A A C T T T G T G T T T T G T T A C C

**NABS I Group C**



# What does this hybridization mean?

- It is unlikely we are inducing the hybridization of *Armillaria* due to disturbance; many individuals have been around for hundreds of years.
- Possible “hybrid vigor”
- May adapt better to changing environment

# Hybridization between NABS X and NABS VII

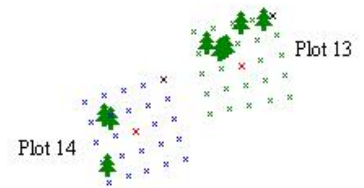
- After DNA analysis, 32 of the 99 NABS X genets are thought to be hybrids between NABS X and NABS VII.
- Some bark fans are associated with this hybrid combination. This may explain why some studies found 5-10% of NABS III, V, VII, and X in pathogenic situations indicated by bark fans from live trees.
- A study from Ida Creek in northern Idaho showed 3% of 951 NABS X isolates to be from bark fans (McDonald et al. 1998). These isolates were collected before we had DNA analysis tools to distinguish NABS X from NABS X hybrids.
- More studies need to be done to determine if NABS X hybrids are solely responsible for low level pathogenicity of NABS X.



# Types of information collected from Nutrition Co-op sites

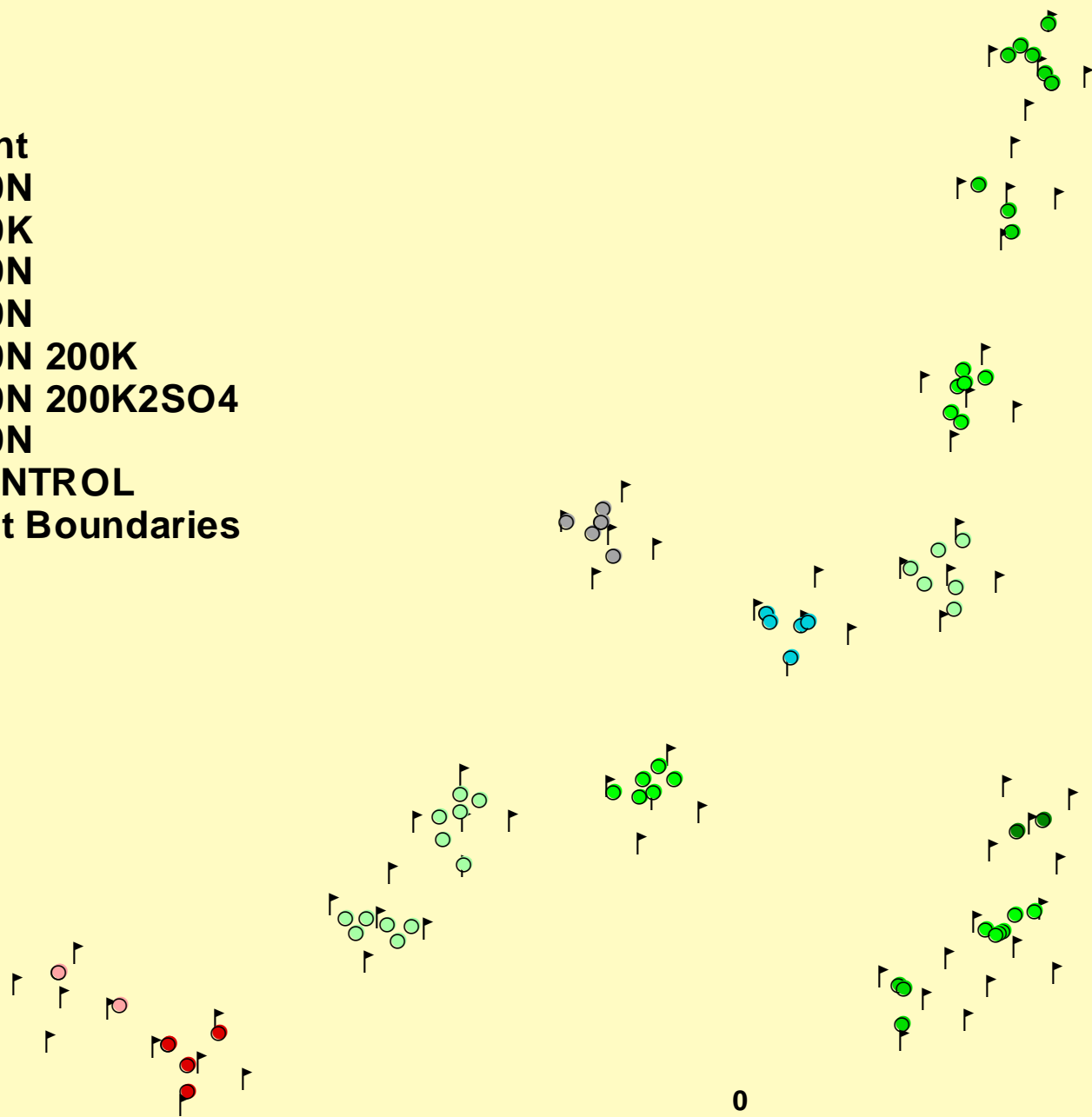
- Isolate ID
- Location
- Host Species
- Collection Type (Bark Fan, Rhizomorph, or Wood)
- Host Status (Dead or Alive)
- Habitat Type
- Fertilization Treatment
- Species ID
- Rock Type

# Soldier Creek 14 Treatment Plots



**Treatment**

- 100N
- 200K
- 200N
- 300N
- 300N 200K
- 300N 200K2SO4
- 600N
- CONTROL
- └ Plot Boundaries



0 0.2 Miles

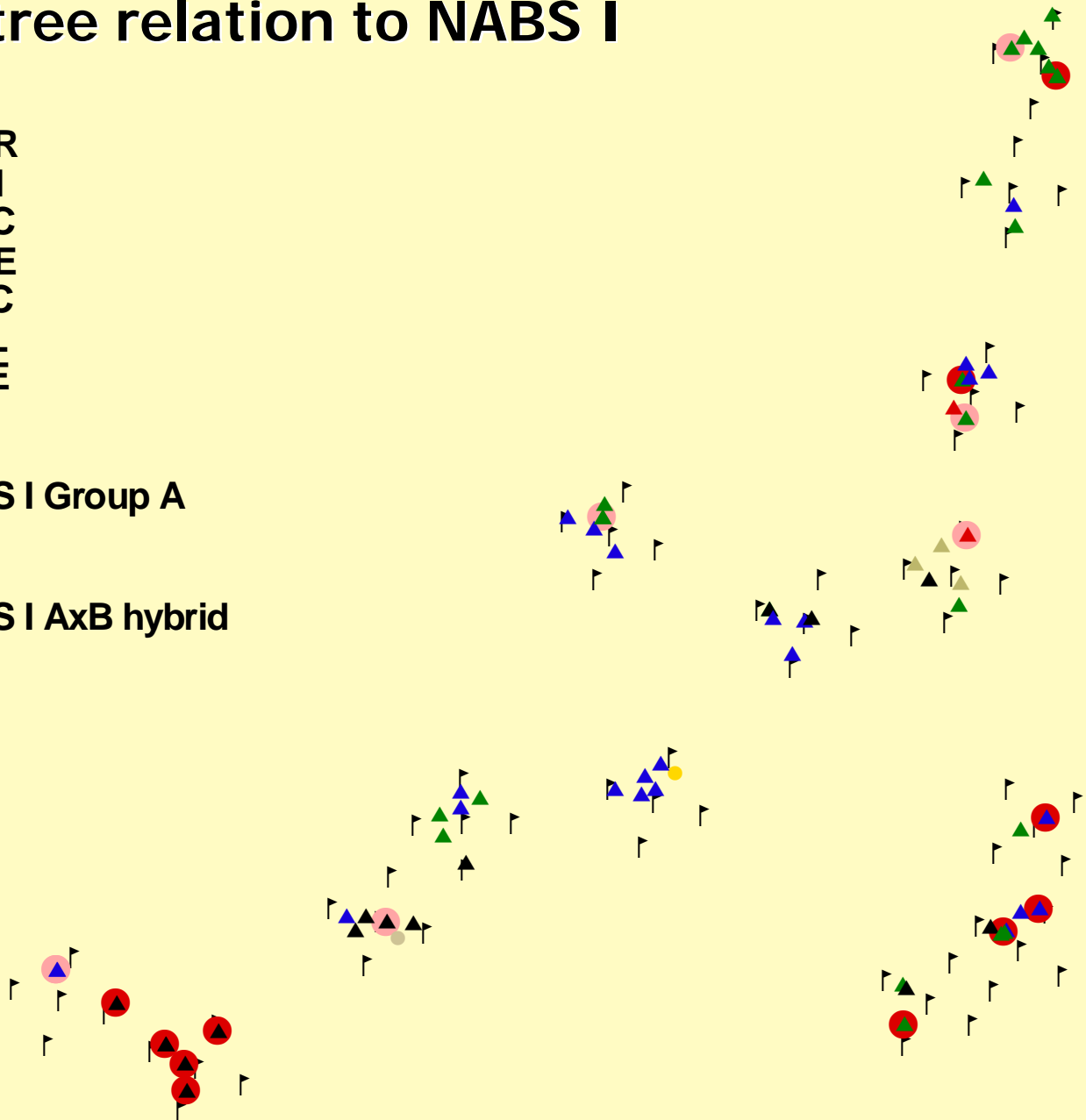


# Host tree relation to NABS I

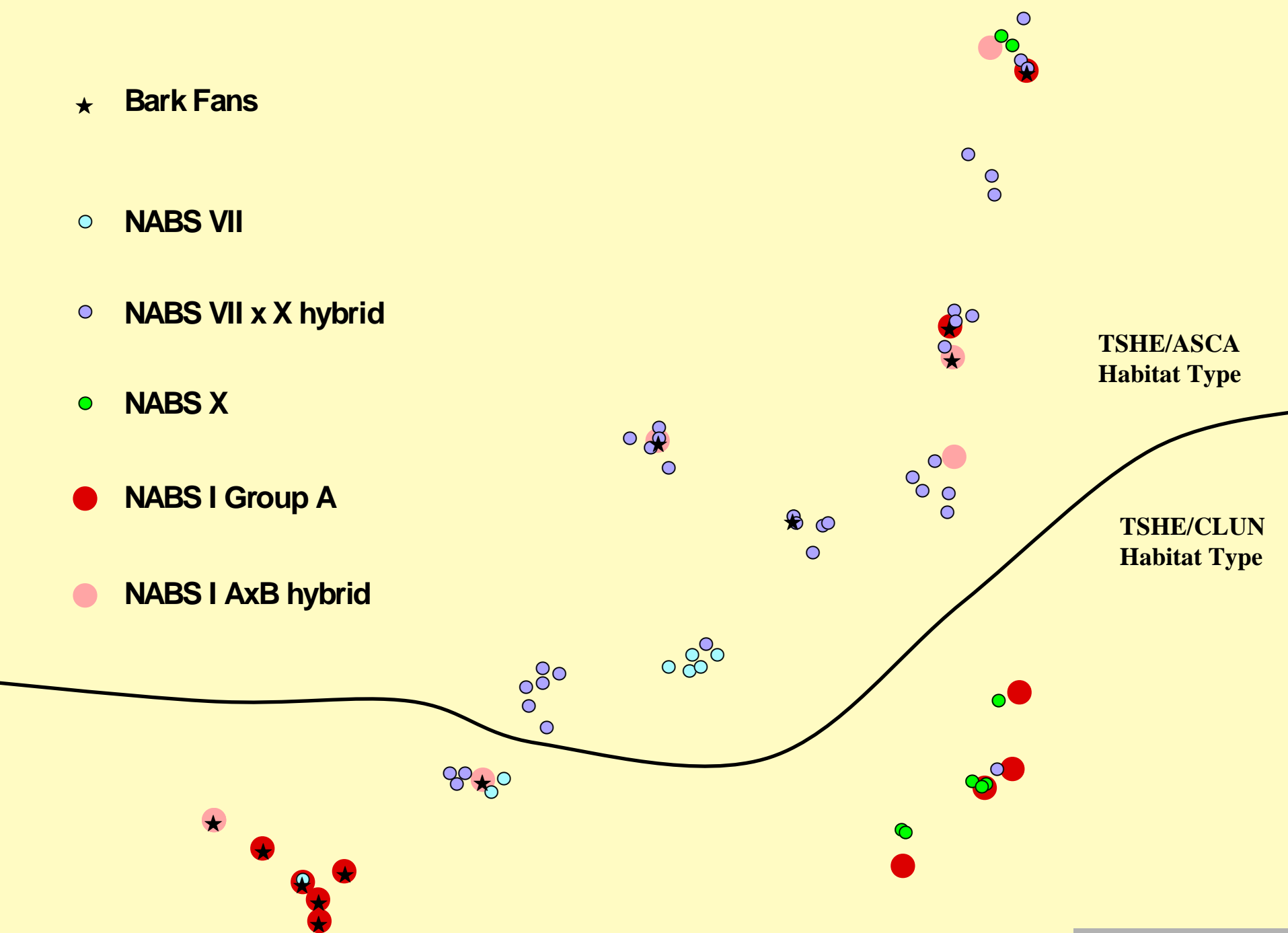
- ▲ ABGR
- HODI
- ▲ LAOC
- ▲ PSME
- SASC
- ▲ THPL
- ▲ TSHE

● NABS I Group A

● NABS I AxB hybrid



- ★ Bark Fans
- NABS VII
- NABS VII x X hybrid
- NABS X
- NABS I Group A
- NABS I AxB hybrid



Each species, hybrid, or grouping of *Armillaria* may occupy different preferred habitat type ranges.

Habitat Type is based on

Moisture

Light

Temperature

 Soil Characteristics

Rock Types



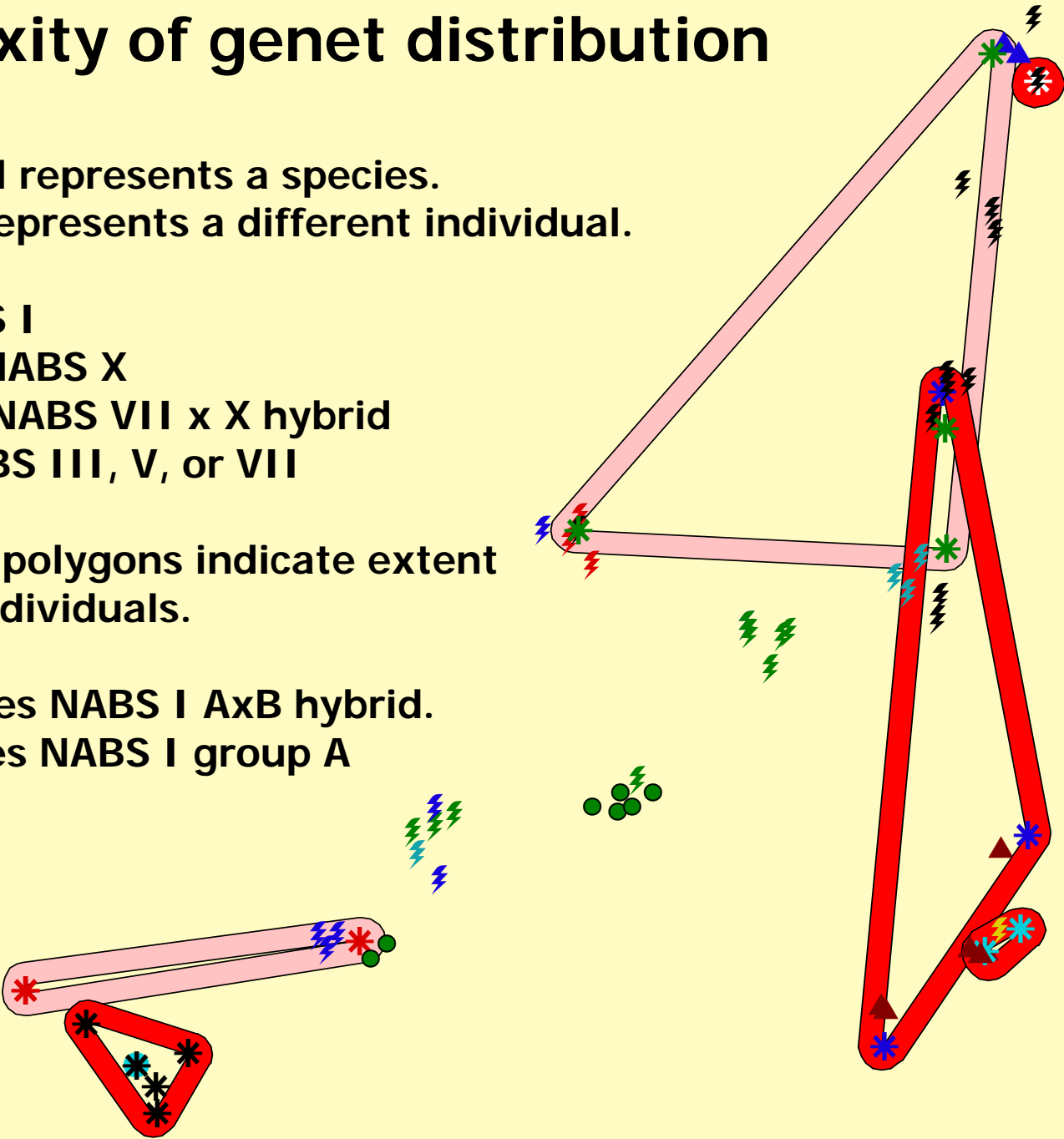
# Complexity of genet distribution

Each symbol represents a species.  
Each color represents a different individual.

- Star = NABS I
- Triangle = NABS X
- Squiggle = NABS VII x X hybrid
- Circle = NABS III, V, or VII

Highlighted polygons indicate extent of NABS I individuals.

Pink indicates NABS I AxB hybrid.  
Red indicates NABS I group A



*Armillaria* interactions with the environment are extremely complex, however with new tools such as molecular genetic techniques and geographic information systems we are in a good position to find answers.

Understanding these interactions will help forest managers learn the conditions leading to mortality and growth loss and will help in management decisions to minimize loss of productivity on *Armillaria* infected sites.

Continued work on the isolates collected from Nutrition co-op plots will begin to yield statistically significant results showing how *Armillaria* interacts with the environment.

# References

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[http://www.glfc.cfs.nrcan.gc.ca/frontline/bulletin\\_no.7-en.html](http://www.glfc.cfs.nrcan.gc.ca/frontline/bulletin_no.7-en.html)
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